Stressful conditions experienced by endangered Egyptian vultures on African wintering areas

M. Carrete¹, G. R. Bortolotti^{2*}, J. A. Sánchez-Zapata³, A. Delgado⁴, A. Cortés-Avizanda⁵, J. M. Grande^{2,6} & J. A. Donazar⁵

¹ Department of Physical, Chemical and Natural Systems, University Pablo de Olavide, Seville, Spain

² Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

³ Department of Applied Biology, University Miguel Hernández, Orihuela, Alicante, Spain

⁴ Laboratorio de Biogeoquímica de Isótopos Estables, Instituto Andaluz de Ciencias de la Tierra, Armilla, Granada, Spain

⁵ Department of Conservation Biology, Estación Biológica de Doñana (CSIC), Seville, Spain

⁶ Centro para el Estudio y Conservación de las Aves Rapaces en Argentina, CONICET, Universidad Nacional de La Pampa, Santa Rosa, La Pampa, Argentina

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Correspondence

Martina Carrete, Department of Physical, Chemical and Natural Systems, University Pablo de Olavide, Ctra. Utrera km 1, 41013 Seville, Spain.

Email: mcarrete@upo.es

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Abstract

Among European breeding birds, those wintering in the Sahel region have undergone a sustained and severe decline. Long-term data show that variation in primary production of the Sahelian staging area significantly affects survival of many species, a relationship probably mediated by trophic resource availability. However, the physiological, hormonal and behavioural responses underlying this relationship remain unexplored. We present a potential explanation for the importance of prevailing conditions during winter to understand the population ecology and current trends of migratory species. We measured corticosterone levels in feathers of Egyptian vultures *Neophron percnopterus* grown in Africa and Europe, showing how conditions faced by birds during wintering periods result in the release of more corticosterone over time than when those individuals were on their summering grounds. This pattern was concordant with home-range size differences (c. 33 times larger in Africa than in Europe). We suggest that as wintering habitat of Egyptian vultures in the Sahel region has degraded during recent times, food availability has also been reduced. An increase in corticosterone during winter with a consequent increase in locomotor activity, for example, food searching behaviour, may normally be adaptive. However, enlarging home ranges could be futile if conditions are not better in the dispersal area, and costs of the higher corticosterone level, including energy expenditure from enhanced activity, may pose a significant trade-off. These physiological responses may be characteristic of other European trans-Saharan migrant birds that have undergone significant population declines.

Introduction

Migratory animals spend different periods of their annual cycle in widely separated and ecologically different locations. Although habitat transformation on the breeding ground has been frequently proposed as the major driver of decline in Palaearctic–Afrotropical migrants, some species appear to be limited by the quantity and the quality of non-breeding habitats (Sanderson *et al.*, 2006). A recent continent-wide analysis of the trends of European breeding birds showed that from 1970 to 2000, populations of Palaearctic–African migrant birds underwent a sustained, often severe, decline (Sanderson *et al.*, 2006; but see Onrubia & Tellería, 2012). The trends of intercontinental migrants were significantly more negative than those of

short-distance migrants or residents, and were largely restricted to species wintering in dry, open habitats in Africa.

Evidence for population regulation through factors at wintering sites is limited, some studies suggesting that it may be significant. Long-term data on migrants that are both short-lived [e.g. sand martins *Riparia riparia* (Szép, 1995) and lesser kestrels *Falco naumanni* (Mihoub *et al.* 2010)] and long-lived [e.g. white storks *Ciconia ciconia* (Schaub, Kania & Köppen, 2005) and Egyptian vulture *Neophron percnopterus* (Grande *et al.* 2009)] show that variation in primary production of the Sahelian staging area, measured as the normalized difference vegetation index (NDVI), significantly affects survival of individuals of all age classes. No causal links between variation in primary production in

Africa and survival have been established, although the authors suggest that the positive covariation of primary productivity (or rainfall) on survival could be mediated by trophic resource availability as food is more abundant in wet than in dry years (Szép, 1995; Schaub *et al.*, 2005; Grande *et al.*, 2009; Mihoub *et al.*, 2010).

Animals respond to unpredictable and disruptive changes in their environment by modifying their behaviour to promote survival. In vertebrates, the rapid activation of the hypothalamic–pituitary–adrenal axis (HPA) results in an increase of circulating glucocorticosteroids [corticosterone (CORT) in birds; Romero, 2004] to mobilize energy stores (Wingfield *et al.*, 1998) and to increase locomotor activity (Astheimer, Buttemer & Wingfield, 1992; Challet *et al.*, 1995), foraging behaviour during migration (Wingfield, Schwabl & Mattocks, 1990), time spent foraging (Kitaysky *et al.*, 2003) and food intake rate (Deviche & Schepers, 1984; Astheimer *et al.*, 1992). These responses allow animals to overcome the negative effects of stressors and re-establish homeostasis in the best possible physical condition (Wingfield & Kitaysky, 2002). However, chronically elevated levels of glucocorticoids have detrimental consequences to cognitive ability, growth, immune defence, body condition, reproduction and survival (Sapolsky, Romero & Munck, 2000; Romero & Wikelski, 2001; Kitaysky *et al.*, 2003; Blas *et al.*, 2007).

Here, we present data showing how a migratory species can experience stressful conditions at their wintering compared with summering grounds and how this influences individual movements. We used the Egyptian vulture as our study model, a migratory species wintering in the Sahel, an ecological and climatic transition zone between the Sahara and the humid tropics of central Africa. Previous studies have shown that besides characteristics in the summering (also breeding) area, environmental conditions faced by individuals during their stay in Africa have a significant effect on survival (Grande *et al.*, 2009). Here, we propose that CORT-mediated incremental increases in home-range size in an ecological degraded area can increase energy expenditure or other costs, which may translate to a negative trade-off. Specifically, we compared the amount of CORT in feathers grown in wintering and summering grounds as well as home-range sizes of non-breeding Egyptian vultures in the two areas. Under this scenario, we expect to find significantly higher CORT levels in feathers grown in Africa as well as larger home-range sizes in this area. Results are discussed taking into account potential alternative explanations.

Materials and methods

Field procedures

We captured non-breeding Egyptian vultures in the Ebro Valley, north-eastern Spain, in June and July 2002–2004 using cannon nets near communal roosts (Grande *et al.*, 2009). Birds were ringed, aged up to the fifth year (adults) by plumage, and bled for molecular sexing. We collected

scapular, primary and secondary coverts from the wings of each individual for corticosterone determination. Egyptian vultures moult their feathers in wintering and in summering regions (Cramp & Simmons, 1980; Forsman, 2007). Thus, we sampled new, brilliant feathers, with non-abraded edges, presumably grown in Africa, as well as older feathers, with faded colour and with worn fringes presumably grown during the previous year in Europe. Very old feathers were not considered to avoid mixing samples of unclear origin. To confirm the origin of the feathers, we used the $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$), related to primary productivity (plant C3/C4), which can be accurately used to determine their origin of growth when the end point are the Sahel and Europe (Cortés-Avizanda *et al.*, 2011). While we cannot be sure of precise discrimination factors corresponding to the vultures in our populations (Robbins *et al.*, 2005), the relatively good fit of our data within the range of values of the vegetation gives us confidence that $\delta^{13}\text{C}$ is correctly discriminating where feathers originally grew (i.e. Europe or Africa; Peñuelas *et al.*, 1999; Sánchez-Zapata *et al.*, 2007). In total, we sampled ≥ 2 feathers in 52 individuals, which were stored in individual envelopes until their analyses in the laboratories.

We obtained information on individual movements as a part of another study where very-high frequency (VHF) and platform terminal transmitters were used to estimate home-range sizes of non-breeding birds in Europe ($n = 33$) and Africa ($n = 14$), respectively. In Spain, vultures were radio-tracked from 2003 to 2005 (range = 2–6 months) using three radio-tracking teams, two located at high points and another one moving by car and intensively searching for birds in a radius of up to 100 km from the roost. Each team recorded the exact hour and the direction of each radio-location to triangulate positions (LOAS available at <http://www.ecostats.com/index.htm>). While in Africa, birds were tracked from 2002 to 2007 (range = 5–15 months) using the Argos satellite system (<http://www.argosinc.com/>). Only LC 0, 1, 2 and 3, with estimated accuracies of c. 9000–1000, 350–1000, 150–350 and <150 m, respectively, were used in the analyses. Conservatively, home ranges were established using minimum convex polygons (MCP) (i.e. the smallest polygon in which no internal angle exceeds 180° and which contains all sites, MCP; Girard *et al.*, 2002). VHF transmitters underestimate home-range sizes when birds move over a large area, so we regularly visited roost sites in the study area and neighbouring regions (250×125 -km area) to identify radio-marked and banded birds (Grande *et al.*, 2009). We never detected our birds outside the study area, so we are confident that the bias does not invalidate the comparison with African ranges.

Laboratory procedures

The sex of the sampled individuals was determined from blood cell DNA using a polymerase chain reaction amplification of the *CHD* genes (Grande *et al.*, 2009).

Stable carbon isotope assays were performed on 0.5-mg subsamples of feather shafts that were combusted in an elemental

analyzer (Carlo Erba 1500NC, Fisons instruments, Milano, Italy) on-line with a Delta Plus XL mass spectrometer (Thermo Finnigan, Bremen, Germany). Analysis of $\delta^{13}\text{C}$ was in triplicate, and the overall precision of analyses was 0.1%. Stable isotope abundance was expressed in δ notation relative to V-PDB (i.e. the Vienna-PDB International Standard Series supplied by the IAEA) as $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ of the sample and standards, respectively. IAEA-CH-6 and USGS40 standards were used to normalize the scale for $\delta^{13}\text{C}$ values according to Coplen *et al.* (2006).

The rest of the collected feathers were used for corticosterone determination. We first measured the length of each feather excluding the calamus, that is, the most proximal part of the feather lacking vane (see Bortolotti, 2010). Then, each feather was cut into pieces and placed in a glass vial with 10 mL of methanol. Vials were placed in a sonicating water bath at room temperature for 30 min, followed by incubation at 50°C overnight in a shaking water bath. Using vacuum filtration, the methanol containing the hormones was separated from the feathers. Vials with the methanol extract were placed open in a 50°C water bath in a fume hood under air until they were completely dry. When the evaporation of the samples was completed, the extract residues were reconstituted with 600 μL of phosphate buffer system (0.05 M, pH 7.6) and frozen at -20°C until CORT measurement by radioimmunoassay. We assessed the extraction efficiency by including feather samples spiked with a small amount (≈ 4000 dpm) of 3H-corticosterone. The coefficient of variation for three different batches ranges from 2.73 to 3.83%. Greater than 92% of the radioactivity was recoverable in the reconstituted samples. CORT values are expressed as a function of feather length (pg mm^{-1}) following Bortolotti *et al.* (2008; 2009).

Statistical analyses

We used generalized linear mixed models (GLMMs) to test whether CORT values (normal error distribution, identity link function) varied among wintering and summering areas. Feather's origin of growth, sex and age were entered as independent variables, while individual and year were included as random terms to account for non-independence in the data. To control for differences in growth rates, and therefore in CORT deposition among feathers (Bortolotti *et al.*, 2008), we included 'type of feather' (i.e. scapular or covert) as a covariate in models. Comparisons of MCP of vultures in Africa and Europe were performed through generalized linear models, including sex, age, area and number of locations (to correct for bias in MCPs associated with monitoring effort) as independent variables.

Results

Feathers were accurately classified as grown in Africa or Europe using $\delta^{13}\text{C}$ (Fig. 1). After controlling for potential confounding effects such as type of feather ($F_{1,42} = 3.15$, $P = 0.0831$) or age ($F_{1,42} = 0.58$, $P = 0.4493$), we found that

Figure 1 Corticosterone (CORT) levels (95% confidence interval) in feathers of male (black) and female (grey) Egyptian vultures *Neophron percnopterus* grown in Africa and Europe. The origin of feathers was determined by using the ${}^{13}\text{C}/{}^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) as C4 grasses (less negative) are much more abundant in the Sahel than in Europe. Graphics represent raw data.

feathers grown in Africa have significantly higher levels of CORT than feathers grown in Europe ($F_{1,42} = 21.35$, $P < 0.0001$; Fig. 1). Indeed, African feathers had 43% more CORT than European ones (8.13 ± 3.06 and 5.65 ± 2.16 , respectively). Females have higher CORT levels than males in both areas ($F_{1,42} = 5.19$, $P = 0.0278$). This full GLMM gave an estimate of ~ 0 for the 'individual' covariance parameter, so we refitted the model by including 'individual' as a fixed factor to detect potential inter-individual differences in the feather CORT pattern observed. The new models showed a significant difference in feather CORT between origin of growth ($F_{1,42} = 25.77$, $P < 0.0001$), without inter-individual differences ('individual' as fixed effect: $F_{49,42} = 1.25$, $P = 0.2323$, or interaction 'individual' $\times$ 'origin of growth': $F_{24,18} = 0.88$, $P = 0.6236$). This suggests that conditions in Africa during feather growth were inducing more CORT secretion than conditions in Europe in all sampled birds (Fig. 1).

In parallel with previous results, and after controlling for the effect of the number of locations, MCP differed significantly between winter and summer (larger in Africa; $\chi^2 = 15.66$, $P < 0.0001$; Fig. 2). Although sample size was small, there is a near significant difference in MCP between sexes (larger for females; $\chi^2 = 3.65$, $P = 0.0562$).

Discussion

Our data show that there was a general pattern for feathers to have higher CORT when grown in Africa, a pattern coincident with that of home-range sizes. Importantly, females have the largest home ranges while in Africa and higher CORT levels than males. Determining causal factors linking these patterns is not straightforward, and experiments, although unlikely to be feasible, are needed. As a first step, it would be enlightening to relate changes in CORT secretion and home-range sizes between wintering and sum-

Figure2 Home-range (95% MCP) sizes (mean $\pm$ SD) of Egyptian vultures *Neophron percnopterus* in wintering and breeding quarters.

mering areas at the individual level, something not possible without our available data. At the same time, efforts should be done to improve our knowledge about how CORT deposits in the different feathers and how and where they grow in order to discard alternative possibilities. Meanwhile, present data are suggestive and merit further research in order to establish its general validity for migratory species.

Vegetation of the Sahel is characterized by an open savannah of spiny acacia trees and annual C4 grasses (the most important source of primary productivity; less negative in $\delta^{13}\text{C}$ values), and because of its aridity, there is a close relationship between rainfall and vegetation growth and, therefore, ecosystem productivity (i.e. NDVI; Niang, Ozer & Ozer, 2008). Long-term studies show that the Sahel has a low and variable rainfall, which has decreased markedly between the early 1960s and mid-1990s, compared with the first half of the 20th century (Niang *et al.*, 2008). This severe drought strongly affected primary productivity and thus richness and abundance of many species (Anadón *et al.*, 2010). The reduced food availability during winter could be perceived as a stressor and, thus, may in part explain the higher CORT levels found in feathers grown during this period (i.e. 43% of increment with respect to mean European feather level). High CORT baseline levels may reflect physiological and behavioural adaptations to maximize fitness in the face of unpredictable environmental events (Wingfield & Kitaysky, 2002). An environment with low rainfall and therefore low ecosystem productivity might induce CORT secretion to increase locomotor activity, that is, food searching behaviour. This 'leave it' strategy is potentially adaptive (Wingfield & Kitaysky, 2002), and poor local habitats and food availability may be overcome by prospecting larger areas (Cote

et al., 2006). However, enlarging home ranges could be futile if conditions are not better in the dispersal area (Niang *et al.*, 2008), and energetic costs of larger movements, or immunosuppression and other negative consequences associated with high CORT levels, are not compensated for (Cote *et al.*, 2006). Actually, we do not know whether costs are compensated or not, or whether the expected costs are the main cause of mortality. Moreover, as individuals differ in their physiological responses to stress (i.e. those physiological responses designed to help an individual survive noxious environmental stimuli; Wingfield & Romero, 2001), birds unable to raise CORT levels and to enlarge home ranges may not survive winter, explaining why individuals with high CORT levels in feathers grown in wintering areas are captured in Europe (i.e. because they survived winter). Finally, it is worth mentioning that current environmental conditions in Africa can be affecting the nutritional status of birds, thus altering feather production (Grubb, 2006) and increasing CORT levels as a consequence of feathers growing at slower rates than in Europe. As we are not able to discern between those possibilities, results should be interpreted cautiously and, as we previously stated, more research is needed to clearly assess the potential role of this mechanisms in fitness components of migratory species.

It is worth noting that the amount of CORT deposited in feathers provides an historical record of an individual's HPA activity during the period of feather growth (Bortolotti, 2010; Lattin *et al.*, 2011). As feathers develop relatively slowly (many days or weeks), these measurements would evaluate a time interval far longer than any other available measure for this hormone, and with the potential to integrate different aspects of HPA activity, including variation in baseline levels and responses to stressors (Bortolotti *et al.*, 2008; Bortolotti, 2010). In this last sense, as stimulated CORT levels are considerably higher than baseline levels, they should have a disproportionate effect on feather CORT deposition during feather growth, thus feather CORT reflecting more certainly the first than the second measure (Bortolotti *et al.*, 2008). Finally, it should be mentioned that baseline CORT concentrations in plasma vary seasonally among birds, with peaks during the breeding season and no discernable differences between migratory and sedentary species (Romero, 2004). Based on the previous evidence, our higher CORT levels recorded in feathers grown in wintering areas could be interpreted as a long-term and retrospective measure of the higher stress suffered by individuals during this period (Bortolotti, 2010), as increased CORT levels in plasma could be detected in feathers (Lattin *et al.*, 2011). In this sense, our data do not allow us to ascertain whether observed CORT levels represent baseline or stress-induced elevations that could compromise physiological functions of birds, so conclusions should be taken with caution (Lattin *et al.*, 2011).

Although we explored one migratory species, the physiological mechanism proposed here is highly conserved among vertebrates (Wingfield & Kitaysky, 2002) and thus could be extended to other European species that have undergone population declines (Sanderson *et al.*, 2006). It is

noteworthy that more than 50% of European birds are trans-Saharan migrants, and many of them show long-term population declines (Sanderson *et al.*, 2006). Indeed, their performance and life-history traits are likely affected by resource availability in their wintering grounds (Gordo *et al.*, 2005), where the lack of protected areas and increasing human pressure result in progressive environmental degradation (Anadón *et al.*, 2010). Indeed, the high human density (Rondinini, Chiozza & Boitani, 2006) and their associated activities such as overgrazing, burning, woodcutting, drainage of wetlands and pesticide use have also contributed to reduce the quantity and quality of resources available for migrant and resident birds (Sánchez-Zapata *et al.*, 2007; Anadón *et al.*, 2010). Future research focused on understanding declines of European species should thus consider physiological mechanisms integrating the experience on the wintering and summering grounds. Moreover, European conservation plans that are currently focused on local-scale events but ignore movements between wintering and summering quarters should be reconsidered, taking into account the importance of multiple-scale management strategies for the conservation of migratory species.

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